Citrus Health Research Forum – Denver, Colorado
June 16-18, 2010

COMPILED REPORT FROM OUTCOME 3 BREAKOUT GROUP

OUTCOME 3: Citrus Groves/Trees Free of HLB and ACP

June 17, 2010 afternoon session:

In the final session, participants discussed various methods of preventing disease and maintaining disease-free status of trees. Discussions surrounded strategies for designing genetically engineered trees that resist HLB infection, various prophylactic treatments that could forestall infection, and the use of various production strategies—from making changes to rootstocks and tree size to the use of flushing mechanisms—that could help protect trees against disease. (*A short version is included below, followed by a more thorough accounting of the discussion.*)

What are researchable elements to address each output: Key deliverables
SHORT VERSION, PRESENTED AT MEETING
Output 1: Genetically Designed Disease-Free Trees

a. Tree resistant to replication of entire complex of HLB-associated bacteria in plant via transgenics and conventional breeding
   i. Standardize screening for resistance/susceptibility: working group has been formed. Procedures will be suitable for screening transgenic and conventional germplasm.
   ii. Exhaustive screening of available germplasm for resistance to HLB.
   iii. Evaluate potential of transgenic rootstocks that can protect non-GE scions.
   iv. Improve methodology for efficient transformation for all important commercial cultivars including transformation of mature citrus tissue.
   v. Identify components of tolerance and/or resistance to HLB/ACP in diverse citrus and relatives.
   vi. Develop parallel model systems to accelerate resistance screening; periwinkle, potato, Arabidopsis as possibilities.
   vii. Develop a transient expression system in citrus to test activity of constructs.
   viii. Produce broader genomic resources and tools to apply toward breeding and host-pathogen-vector interactions.
   ix. Develop and evaluate components of high-throughput breeding; short cycle plants, horticultural practices to accelerate flowering, generation of markers for resistance etc.
   x. Produce high-throughput phenotyping methodology, applicable to conventional breeding as well as transgenic approaches.
   xi. Develop mutagenized lines of citrus for screening for HLB/ACP resistance and other functional genomics applications.

b. Tree tolerant to replication of entire complex of HLB-associated bacteria in plant. No difference in path to resistance or tolerance. Tolerance is the consolation prize……..

c. Tree resistant or repellant to ACP via genetics.
   i. Exhaustive screening of available germplasm to identify material resistant to psyllid feeding and development.
   ii. Explore and identify GM solutions for ACP resistance: transgenes that affect/interrupt movement of CLas into the gut or prevent CLas attachment to the vector: lectins; RNAi strategies; Bt; Vip (precursor to Bt).
   iii. Produce trap plants that kill psyllids, GE border plants with no risk of pollen flow.
   iv. Develop high-throughput methods to explore host-vector interaction and vector-bacterium interaction.
   v. Utilize "–omics" approaches to understand and disrupt host-vector-bacterium interactions.

Output 2: Prophylactic treatments to prevent infection or ACP-infestation of tree: (Not Prioritized)

i. Discovery of small molecules that specifically target CLas and are delivered through specific routes.
   a. Antibiotics.
   b. Compounds screened from combinatorial libraries.
ii. Discovery of small molecules that can be sprayed to protect against ACP.
   a. Antibiotics (focusing on gut endosymbiants critical for ACP).
   b. Volatiles.
   c. Compounds screened from combinatorial libraries.
iii. Methods to deliver small molecules:
   a. Viral vector.
   b. RNAi delivered via vector, rootstocks or transgenic barkpatch or direct uptake.
   c. Nanoparticles, microencapsulation using lipids and their delivery and attachment to other compounds or chemicals.
   d. Means of delivery of therapeutic compounds from rootstock to scion. RNAi can be delivered and complexed with proteins in rootstock (or through inarching).
iv. Develop transgenic or other Las/ACP suppressive endosymboints.

Output 3: Change the production system or tree physiology: researchable areas (Not Prioritized)

i. Investigate impact of growth regulators on host resistance.
ii. Develop systems to use dwarfing rootstocks for intensive plantings under screen.
iii. Control of tree flushing (PGRs, nutrition, irrigation, root-pruning).
iv. High density planting, tree architecture, and grove architecture to minimize edge effects, etc.
v. Early flowering, precocious bearing scions, and rootstocks that impart early high-quality fruit.
vi. Growing citrus on trellis, scaffolding and fruiting walls.
vii. Better ACP control with high density planting and smaller trees.
viii. Additional systemic insecticides to protect trees until later in orchard life (limited Admire/acre).

ix. Protect replants with clay or netting (repellant or insecticide impregnated) to maintain them until bearing.
x. Interplanting with guava.
xi. Transgenic push-pull system. (attract and bait, repel)
xii. Minimization of stress on young trees through nutritional programs irrigation and other management practices, to reduce infection rate. Anecdotal evidence for this.
xiii. OR… Can you reduce infection/disease thru stressing plant? Compare and contrast to generous delivery of nutrients and water.
xiv. Rootstock effect on disease progression thru surveys of existing planting and new controlled trials in field and greenhouse.
xv. Can nutrition/other factors be managed to enhance regeneration of phloem? Demonstrated that in Arabidopsis transgene can influence conversion of xylem to phloem.
xvi. Alternative machinery needed to manage new types of plantings.
xvii. Early cropping as influenced by interaction between girdling (scoring of cambium): and rootstock vigor.
xviii. Removing summer flushes, as is done in China, to reduce ACP feeding. Perhaps done through growth regulators.
xix. Effective cold protection (e.g. removable floating row covers) to confidently grow trees in N end of citrus areas with lower ACP pressure.
xx. Wind machines to move ACP out of grove.

Communication activities:

- Develop database as a resource to researchers, describing experimental details and results following challenge of diverse germplasm with CLAs; both transgenic and conventional.
- Develop venue for posting details of recent research results, e.g. CRDF Web site could host abstracts when submitted for professional societies.
- Mechanism to share IP to expedite moving genes into new cultivars and developing new cultivars, so all can benefit from development of new varieties.

LONG VERSION; Outcome group 3. (there is e redundancy with short version)

Output 1: Genetically Designed Disease free trees
understanding of components of tolerance and/or resistance to HLB/ACP in citrus, including an interaction of genetics and nutrition.
Mutagenized germplasm in citrus is difficult. Need ideas on how to develop these products.
Transgenic rootstock that can protect variety of scions

a. Tree resistant to replication of entire complex of HLB-associated bacteria in plant
    i. Two major strategies: transgenics and conventional breeding

First step: Standardized screen for resistance (or for susceptibility); working group has been formed; would apply to both types of resistance development. Some overlap across conventional and transgenics development research will be working together and communicating.
How will you know that you have something? If screening system was 90% reliable within ? months (perhaps longer; up to a year or more). How to prove it

is successful? Three to 4 groups that are using it across environments. Even if plant remains healthy, will not prove that it is resistant.
Short term; can ID things that are susceptible and rogue them out. **Call it screening for susceptibility rather than for resistance.**

Note: *Criteria for susceptibility will be determined by committee. Time frame: proposed standard protocol and then test it/validate in several labs. Within 6 months can have a proposed method that can be initiated at multiple locations (tbd). Will include source of inoculum (RL), sources of germplasm, non-transgenic selections for measure of background, psyllid inoculation (David Hall to be consulted), etc. Better to test genes in particular genetic background or go for important commercial cultivars. For Plumpox virus, protocol was published in an international journal made available to numerous partners; can be a deliverable.*

i. Database to describe what has been challenged with Lib; both transgenic and conventional. Genes and germplasm, promoters, signal peptide to be tested: Some genes till target the Lib but others will target the host defense response (enhance or limit host response). CRDF website could have this capacity where abstracts could be archived (CHRP website for inventory is a possibility). Can a venue for reporting new methods is needed, prior to publication. Quarterly reports are provided, although these are not always a reliable source of information. Monthly conference call was suggested for very focused activities.

ii. Improved methodology for efficient transformation for all important commercial cultivars and to encompass mature transformation.

iii. parallel model systems to accelerate screening; periwinkle, potato, Arabidopsis as possibilities

b. Tree tolerant to replication of entire complex of HLB-associated bacteria in plant. Is this acceptable? Is true resistance possible? Tolerance may be the more acceptable product. Need to consider needs of entire national/global industry. No difference in path to resistance or tolerance.
    i. Tools to pursue conventional breeding to achieve these ends. Need broader genetic resources and range of germplasm, high thru-put SNP platform, apply genomics to breeding programs. High thruput breeding; short cycle plants, horticultural practices to accelerate flowering, ID markers generated. High-thruput phenotyping.. Applies to conventional breeding as well as transgenic approaches.

c. Mechanism to share IP to expedite moving genes into new cultivars and developing new cultivars, so all can benefit from development of new varieties. This will involve germplasm managers across institutions and industry. IP offices and OTT should be talking. Need a working group to get discussion started. Gail will start discussion with respective offices, and will report back to citrus group 1.

d. Tree resistant or repellant to ACP via genetics. Again similar pathways/tools as described previously, but screening will be different. Need entomologists to handle this piece. Extensive list of gaps from morning discussion.
    i. Products that affect/interrupt movement and/or attachment of the Lib in the gut of the vector: lectins, protein attachment to gut, RNAi strategies, Bt's, Vip's (precursor to Bt), exhaustive screening of available germplasm to resistance to psyllid feeding and development. Can someone be contacted from mosquito research to provide insight?
    ii. Trap plants, border plants with no risk of pollen flow.
    iii. High-thruput methods to explore host-vector interaction and vector-bacterium interaction.
    iv. "–omics" approaches to approach host-vector-bacterium interactions.

Output 2. Prophylactic treatments to prevent infection or ACP-infestation of tree: gaps identified.

i. Discovery of small molecules that specific target Lib and delivered thru specific route. (depends on what is accomplished in output 1).
    a. Antibiotics
    b. volatiles
    c. Screen from combinatorial libraries
ii. Discovery of small molecules that can be sprayed to protect against ACP.
    a. Antibiotics
    b. volatiles
    c. Screen from combinatorial libraries
iii. Methods to deliver small molecules:
    a. Viral vector
    b. RNAi delivered via vector, rootstocks or transgenic barkpatch or direct uptake.
    c. Nanoparticles, microencapsulation using lipids and their delivery and attachment to other compounds or chemicals
    d. Means of delivery of therapeutic compounds from rootstock to scion, as in grape. RNAi can be delivered and complexed with proteins in rootstock or through inarching, as a low-profile entry point for prophylactic compounds.
iv. Transgenic or other Las/ACP suppressive endosymboints; are there any in phloem from healthy trees? Hard to find even in diseased plants. Ping has some evidence that there are some Libs that are not as pathogenic as others. Low priority, as part of other research.

Output 3: Change the production system or tree physiology: researchable areas

i. Impact of growth regulators on host resistance.
ii. Dwarfing rootstocks under screen
iii. Control of flushing
iv. High density planting, tree architecture and grove architecture to minimize edge effects

v. Early flowering, precocious bearing scions and rootstocks that impart early high-quality fruit.
vi. Growing citrus on trellis, scaffolding and fruiting walls.
vii. Better ACP control with high density planting and smaller trees.
viii. Additional systemic insecticides to protect
ix. Protect replant with clay or netting (repellant or insecticide impregnated) to maintain them until bearing.
x. Interplanting with guava
xi. Transgenic push-pull system. (attract and bait, repel)
xii. Nutritional programs, irrigation and other management practices. minimization of stress on young trees with relation to infection rate thru nutritional programs. Anectodal evidence for this.
xiii. OR…Strict delivery of nutrients to help trees "hold on" to fruit! Can you reduce infection/disease thru stressing plant? Compare and contrast to generous delivery of nutrients and water.
xiv. Rootstock effect on disease progression thru surveys of existing planting and as new rootstocks come on board relative to the rate of infection with HLB.
xv. Can nutrition/other factors be managed to enhance regeneration of phloem? Some things done in Arabidopsis converting PH to XY.
xvi. Alternative machinery needed to manage new types of plantings. Adaptation needs to be designed.
xvii. Girdling (scoring of cambium): how it relates to rootstock vigor. Thought to regenerate from vegetative to fruit production; shift in physiology.
xviii. Removing flushes, as is done in China, to remove favorite food source for ACP feeding. Could be done through growth regulators. Can use this to time use of insecticides.
xix. Root pruning: how does it impact HLB/ACP? Apples; controlled tree growth. Can this control flushes? Factor of open hydroponic system where roots are concentrated beneath water delivery.
xx. Effective cold protection under screen to avoid ACP.
xxi. Wind machines to move ACP out of grove.